MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0
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Product name: Pfu DNA Polymerase

Cat #: AD-200, AD-205, AD-210, AD-OEM

Description:
Pfu DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum Pyrococcus furiosus. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'->3' direction. Pfu DNA Polymerase also exhibits 3'->5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5'->3' exonuclease activity. The main difference between Pfu and alternative enzymes is Pfu's superior thermostability and 'proofreading' properties. Unlike Taq DNA polymerase, Pfu DNA polymerase also possesses 3'->5' exonuclease proofreading activity, resulting in PCR fragments with fewer errors than Taq-generated PCR inserts. Pfu DNA polymerase is efficient for techniques that require high-fidelity DNA synthesis, but can also be used in conjunction with Taq polymerase to obtain the fidelity of Pfu with the speed of Taq polymerase activity.

Supplied with:
10x Pfu Reaction Buffer (with dNTP)

Supplied in:
20 mM Tris-HCl (pH 8.0)
40 mM NaCl
2 mM Sodium Phosphate
0.1 mM EDTA
1 mM DTT
Stabilizers
50% (v/v) glycerol

Unit Definition:
One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTPs into acid insoluble material in 30 minutes at 74°C under standard DNA polymerase assay conditions.

Protocol:
1. Add template DNA (10 pg – 1 ng for plasmid and 0.1 – 1 µg for genomic DNA) and both forward and reverse primers (200 nM of each final concentration) to the PCR tube.
2. Add 10x Pfu Reaction Buffer to 1/10 of final volume, add nuclease-free water to bring the total volume to final volume.

Component	Volume	Volume
Template DNA	10 – 50 ng	10 – 50 ng
5' Primer (5 µM)	1 µL	2 µL
3' Primer (5 µM)	1 µL	2 µL

10x Pfu Reaction Buffer (with dNTP)	2.5 µL	5 µL
Pfu DNA Polymerase	1 µL	2 µL
dH_2O	To 25 µL	To 50 µL
FINAL VOLUME	25 µL	50 µL

3. Mix the PCR mixture thoroughly and spin down briefly.
4. Place the PCR tubes into the PCR machine and start the PCR reaction.
5. Load 2 µL or 5 µL reaction mixture directly on agarose gel to check the result after PCR reaction. Load the rest on agarose gel if the amplified fragment need to be gel purified for downstream experiment.